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NEWCOMBE, F.C.

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the growth of plant tissues. 1893.

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THE EFFECT

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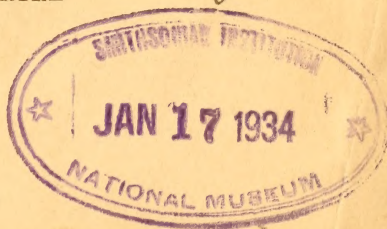
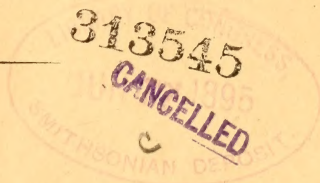
BY

FREDERICK CHARLES NEWCOMBE B. S.

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Effect of mechanical
resistance on the growth of
Plant tissue/

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INTRODUCTION.

It has long been known from the investigations of Mirbel¹, Link², Treviranus³ and Meyen⁴ and the somewhat later researches of Schleiden⁵, Kützing⁶, Unger⁷ and Frank⁸ that many hollow organs such as the leaves of *Allium cepa* and the pith of many plants during primary growth indicate the beginning of cavity-formation by the separation of cells from one another, after which, as the radius of the organ increases, the cavity enlarges by the death of the innermost cells and the enlargement and outward movement of the more external.

The question whether organs which thus become hollow would become hollow if this first separation of cells from one another were prevented, or in other words, *whether the cells normally destroyed in such cavity-formation would die if the cells were not pulled apart by surrounding tissue* has never been made the subject of experiment. The work here presented is in part an attempt to solve this question.

The method pursued in the study of the subject just stated, has brought results bearing also on another question which includes the former and in which important conclusions have been reached by de Vries and Krabbe, — *the effect of radial pressure on the growth of tissues in general*.

De Vries⁹ in his experiments to determine the relation of normal cortical pressure to the formation of the annual rings of trees,

¹ Traité d'anatomie et de physiologie végétales. Paris 1802.

² Grundlehren der Anatomie und Physiologie der Pflanzen. Göttingen 1807.

³ Beiträge zur Pflanzenphysiologie. Göttingen 1811. — Physiologie der Gewächse. 1. Bd. Bonn 1835.

⁴ Neues System der Pflanzen-Physiologie. 1. Bd. p. 313. Berlin 1837.

⁵ Grundzüge der wissenschaftl. Botanik. 3. Aufl. 1. Theil p. 247. Leipzig 1849.

⁶ Philosophische Botanik I. p. 316.

⁷ Anatomie und Physiologie der Pflanzen p. 201. Pest, Wien und Leipzig 1855.

⁸ Beiträge zur Pflanzenphysiologie. Leipzig 1868.

⁹ De l'influence de la pression du liber sur la structure des couches ligneuses annuelles. Extrait des Archives Néerlandaises 1876. Also in Flora 1876. p. 2.

wound various branches and stems with twine, and in the results given by the microscopical examination at different periods of subsequent growth he enumerated the 3 following pertinent principles:

1. The growth of the wood elements in the radial and in the tangential direction is influenced by the pressure under which it takes place; the greater the pressure the more feeble will be the expansion.

2. The number of successive divisions in the cambium depends on the radial pressure then present; other things being equal, the greater this pressure, the fewer will be the divisions in a radial series.

3. The proportion of the number of vessels to the number of wood-fibres in a ring depends on the pressure under which the ring has been formed; the greater the pressure the less favorable is the proportion to the number of vessels.

Krabbe¹ by a peculiarly constructed chain working over brass strips laid on the bark of trees was able to apply a graded and definite radial pressure. His chief result was the proof that normal cortical pressure does not effect the building of the annual rings of trees. Aside from this he obtained some results which bear directly upon work recorded in the present paper. These results however include numbers 1 and 2 given above as already contributed by de Vries. In addition, Krabbe found

1. That in trees of neither Conifers nor Dicotyledons would the cambium cells under any amount of pressure divide at a smaller size than normal.

2. That in Conifers a pressure of from 3 to 5 atmospheres, in Dicotyledons of 5 to 7 atmospheres is required to lessen either in number or size the elements arising from the cambium.

3. That from the pressure last named growth would proceed up to a pressure of 10 atmospheres in Conifers and 15 in Dicotyledons, but would proceed more slowly.

4. That in dicotyledonous trees as soon as the pressure had passed a certain height — usually 10 atmospheres — an active cork meristem was formed in the cortex.

The work of which the following pages are a record was done in the winter semester of 1892 and the summer semester of 1893, in the Botanisches Institut at Leipzig, under the direction of Professor Pfeffer, for whose able guidance and kindly bearing the Author feels himself greatly indebted.

¹ Ueber das Wachsthum des Verdickungsringes und der jungen Holzzellen in seiner Abhängigkeit von Druckwirkungen. Berlin 1884.

I. METHODS.

In the present work the effort has been to arrest the radial and longitudinal extension of primary growth, by the application of mechanical resistance with as little injury to the plant as possible. The means to effect this end has been the application of gypsum casts¹.

When a plant organ was to be encased an envelope of stiff paper was fitted around the object, and a thick mixture of gypsum and water poured into the mould. The diameter of the cast varied according to the size of the organ encased, but was nearly always of such a size that it needed support other than that given by the plant, since experience taught that casts several times the diameter of the organ encased might be sprung.

The length of the plant segment enclosed in gypsum has varied between 3^{cm} and 10^{cm}. A shorter cast than 3^{cm} could not be depended upon to give intelligible results, since it had been found that near the limits of the cast the behaviour of the part enclosed is quite different from what it is well within. It is certain that by confining the growth of an organ for the length of but a centimeter or two, results would be obtained very different from those here presented.

To control the results found in the parts grown within casts, corresponding parts have been kept within opaque wrappings, and numerous comparisons have been made with normal plants.

II. EXPERIMENTAL PART.

The objects for the experiments have with 2 exceptions been stems, and in the majority of cases such stems as become hollow during primary growth. The structure which the organs have had

¹ Pfeffer has used this method in researches for some time and has described it in *Berichte d. k. sächs. Gesellsch. d. Wissenschaften*, December 1892: Ueber Anwendung des Gypsverbandes für pflanzenphysiologische Studien.

at the time of enclosing them in gypsum has been determined by examining for each case several stems of like development.

Some objects submitted to experiment have been used for one purpose, some for another; the peculiar features of each will be found noted with the description under the respective plant species.

Juncus effusus L.

Five casts were applied to the bases of stems of *Juncus effusus*, each cast including several shoots. Some shoots projected above the gypsum, and others were wholly covered. Examinations were made after 4 weeks and again after 11 weeks. Those shoots that had projected well out of the gypsum had made farther growth, the cast not preventing elongation. Those that were wholly covered by the gypsum remained during the 11 weeks living and almost unchanged. The only alteration that could be found in the latter was a growth of peripheral cells toward the centre, forming thus a zone of living cells wider than normal, and crushing the previously existing dead cells together in the centre.

Allium cepa.

Several experiments were made by including within a cast the young leaves and the upper part of the bulb of a germinating onion, then securing the cast by cloth and twine bands to the bulb so as to prevent cast and bulb from separating. The longest period that such a cast was allowed to remain was 31 days.

The results were the same in all the preparations. All parts of the leaves remained alive, the peripheral cells elongating toward the centre and thus filling up the previously existing intercellular spaces there. The bulb itself remained turgid, and on removal of the cast growth was resumed in the same leaves that had for weeks made no enlargement.

The fact that the spongy parenchyma filling the middle of the very young leaves in *Allium cepa* is replaced by a large cavity in the mature leaves is familiar to all. Sachs¹ long ago found that when the leaves were grown in darkness they did not become hollow.

¹ Sachs, Ueber den Einfluss des Tageslichtes auf Neubildung und Entfaltung verschiedener Pflanzenorgane. Botanische Zeitung 1863. Beilage.

Observations show however that the statement of Sachs needs modification. Twenty to twenty-five etiolated leaves have been examined, with this result: The stronger etiolated leaves become hollow; only the weaker ones remain solid.

The different structural relation in the normal, hollow leaves and in the etiolated leaves with no cavity or with a small cavity are not difficult to trace. In the very young leaf, even down to the bulb near its origin, the middle mass of parenchyma contains large intercellular spaces, while the peripheral cells are very small and the horizontal radial and tangential diameters are equal. As the young leaf elongates and increases in thickness, the intercellular spaces in the middle tissue increase greatly in size, the cells separating so as to form a network. As the leaf grows up into the light, the peripheral cells enlarge greatly in every direction, forming the well-known H-palisade assimilating cells and large intercellular spaces. The middle parenchyma does not grow so rapidly, and the strain breaks the network. The middle cells are however living at the time and live for some time after they are split apart. The cells that have the smallest surface connection with the neighboring tissue are the first to die.

In the etiolated plants the peripheral cells had grown but little in size and had preserved the form which they had within the bulb. Thus there was no inflation here as in the normal leaves, but in cross-section the leaves had the form of an ellipse or meniscus. In the larger leaves however the growth of the periphery was too great for that of the central parenchyma, and some splits took place followed by collapse of cells. Where only a few centimeters of a leaf are kept in the dark, the etiolated part will grow enough to form a cavity.

The formation of a cavity in a short segment of a leaf may be prevented by the application of gypsum casts, though here of course etiolation as well as pressure is effected. There are two difficulties however connected with the successful use of gypsum. In the first place the leaf of *Allium* is so smooth that it will grow out of the cast. In the second place the meniscus-shape of the cross-section of the leaf at the time when the cast must be applied usually allows the infolding of the flat side as the pressure by growth increases. The result of this infolding of the strong periphery is the stretching and crushing of the weak parenchyma in the middle. In 10 leaves around which casts of 3^{cm} length were kept for 5 weeks, the flat side had in each case folded in, and each leaf had a mass of dead cells in the middle, though the number of rows of living hyaline

cells separating the dead mass from the green peripheral cells was always from one to four greater within the cast than above or below it.

Another leaf from which the cast was removed after an application of 18 days showed no infolding, yet all peripheral cells had greatly elongated toward the centre and some of the weak cells there were crowded together. Except these few collapsed ones all of the central, hyaline cells, 6 to 8 in a radius were living, while above and below the cast were but one or two rows of similar cells.

Two casts were now applied in a manner different from the preceding, the first to compel less longitudinal growth within the cast, the second to check growth at an earlier stage than had been done. For the first purpose, the leaf was made to take a zigzag course through the cast by thrusting pins through the paper mould before the gypsum was poured in and thus bending the leaf back and forth. For the second experiment, the scales of an onion were so cut away above that a cast could be applied 2^{cm} lower than previously done. The first cast remained 13 days, the second 15 days, the leaves in question carrying the cast up from the bulb and attaining in this period each a length of 15^{cm}. This time was taken to end the experiments since the leaves then showed the beginning of the infolding at the upper limit of the cast.

Removal of the casts and sectioning showed in each leaf a solid mass of living tissue, — no dead cells anywhere. The central mass of parenchyma cells did not show the spongy condition which it had when the casts were applied. The cells had been crowded closely together by growth toward the centre. Above and below the casts the leaves had their normal cavities.

The changes in tissue or cells induced by the casts were marked everywhere by the incomplete development of the assimilating cells, and by the abnormal radial elongation of the 2 inner rows of the same tissue. In the normal the mechanical tissue is but slight and no change was here evident within the casts.

Equisetum limosum L.

In addition to what Frank¹ has written on the formation of the intercarinal canals in *Equisetum*, a few words may be given as to the time of this formation.

¹ Beiträge zur Pflanzenphysiologie. Leipzig 1868.

The young shoots of *E. limosum* when 5^{cm} or 6^{cm} above the earth will show only the central canal and those in the bundles; the intercarinal canals have not been formed. The latter are however at this time present in the stalk at the level of the earth or just below and progress gradually upward, the nearness of the approach to the apex being exceedingly variable. Some stems may have no intercarinal canals from the apex down through the distance of a dozen internodes.

Five shoots not more than 5^{cm} in height were enclosed in gypsum to within a short distance of the growing point. 21 days afterward when the stems had reached a height of 20^{cm}, 2 of them were sectioned. The growth in thickness outside of the cast had been slight, but the casts had prevented the internodes which they inclosed from elongating. One plant showed living cells in the place of intercarinal canals within the cast while such canals were present above and below, and the other plant which was a strong one showed intercarinal canals smaller within the cast than above and below.

Forty-four days after the casts were applied the other three plants were examined. They had grown to a height of about 40^{cm} without growing much in thickness. All of them showed intercarinal canals present within the limits of the casts. Moreover structural differences within and outside the casts were not to be seen. In such a stem where there is so much free space internally, where there has been scarcely any growth in thickness, and where the longitudinal elongation would have been small, there cannot have been much unusual pressure called forth.

Petiole of Ricinus communis L.

The petiole of *Ricinus communis* becomes hollow before elongation is complete, the schizogenous beginning of the cavity being soon followed by the collapse of cells.

Parts of 2 petioles before any dead cells or cavity were present were enclosed in gypsum. One grew subsequently for 26 days, the other for 45 days before being cut for examination. The changes were alike in both. Within the casts one had in the cross-section 3 or 4 dead cells at the centre, the other 10 dead cells; but neither had a cavity. The multiplication of elements in the bundles had been about half within the casts what it was outside, and the thickening of cell-walls in phloem, xylem and at the inner ends of the bundles was less than in normal parts of the same leaf-stalks.

Thus within the casts nearly all of the «pith» had been preserved while outside nearly all was dead and collapsed.

Triticum repens L.

The rhizome of *Triticum repens*, which forms a narrow central cavity, presented itself as a favorable object for experiment. None of the many rhizomes examined has shown the presence of a cavity earlier than 6 weeks after the beginning of growth in the spring. In this time the sclerenchyma zone becomes so thick that neither radial nor longitudinal displacement can be thought of as the immediate cause of the death of the cells. In the centre of the rhizome, the cell-membranes, hitherto seemingly strong, become wrinkled and separate, and the collapse of cells forms the cavity.

Casts were put around young internodes of rhizomes that had reached a length of not more than 3^{cm} or 4^{cm}, the stem being exposed by cutting away the sheathing leaves. Thus 4 rhizomes were encased when the internodes had reached less than half the length which the free neighboring internodes subsequently attained. After an interval of 53 days the preparations were examined. One had died, one rhizome had not grown, and two had grown greatly.

The rhizome that had not grown but was still living, showed a healthy appearance within, the cells being apparently in the condition in which they were when the cast was applied. The 2 rhizomes that had made good growth showed in the internodes within the casts as said above less than $\frac{1}{2}$ the length of neighboring free internodes, and a diameter 10% narrower in one, 25% narrower in the other than in the same rhizomes outside of the casts.

Within the internodes surrounded by gypsum the development of cells was in cross-section normal except that the parenchyma cells were smaller than usual. The mechanical tissue was as well developed in the distal end of the internodes within as outside the casts. Here although the internodes in question were prevented from reaching $\frac{1}{2}$ their normal size, and it is certain that cells not normally extended reached the permanent stage, considering that the growth is intercalary the cells may not have been so very far from their full form when the casts were applied.

In both these rhizomes that grew well there was a cavity above and below the cast. Within the cast the preparation with the smaller diameter showed no trace of cavity-formation, in the other a cavity was just beginning.

Zea mais L.

From a young plant of *Zea mais* that had several lower internodes fully extended the leaves were cut away so as to expose several of the upper internodes when each was not more than a centimeter in length. About these internodes a gypsum cast was placed, and the plant allowed to grow farther. At the end of 37 days the plant was cut for examination. Several internodes had elongated above the cast and a good growth had been made. The diameter of the stem above the cast was no greater than that within.

The microscopic examination of the internodes that the gypsum had not allowed to elongate showed there much weaker bundle-sheaths than above and below; the usual thick-walled and lignified peripheral zone of fundamental tissue was absent, though present in the normal parts of the same stalk. Within the cast the air-canal in the bundles had not been formed; the surrounding thin-walled cells could be seen in cross-section to connect directly with the vessel which is normally destroyed in the formation of the air-canal.

Four other plants of this species when the internodes were all very short and not more than 3^{cm} or 4^{cm} above the earth were surrounded by gypsum at the level of the earth without removing the leaves. The plants thus grew in length within the casts but not much in thickness. One plant was examined after a lapse of 23 days, the other 3 after 60 days. All grew as well as normal ones in the same pots, the older plants reaching the blossoming stage and being a meter in height. The enlargement of the stem above the casts was not sudden but very gradual, extending over one or more internodes. The thickness of the stems within the casts was probably two thirds of what they would have been normally.

Three of these plants showed the normal amount of mechanical tissue present within the casts. Cells of all kinds and vessels were of smaller cross-section within than above and below the casts. The fourth plant was one of those that grew for 60 days after casting. The difference in diameter between the part in the cast and that without was greater here than in any of the other three. In its histological structure it showed less mechanical tissue in the constricted part than above and below, though the difference was not so great as present in the first plant described where the internodes had not elongated.

Caltha palustris L.

Cavity-formation in this plant is very similar to that in the leaves of *Allium cepa*. In the young stem before half elongated the cortex and pith are composed of plates of cells separated by large schizogenous canals giving in cross-section a net-like appearance. As growth progresses, the net work in the pith suffers many rents, the separating cells living for some time after they have been torn apart. By this process of separation some cells are left with but small connection with their neighbors and such are the first to die and collapse. In this partly schizogenous, partly lysigenous way the cavity grows till it arrives within a few cells of the bundles. A central cavity is present about the time that elongation ceases.

Gypsum casts were applied to 4 stalks, in 2 cases before any dead cells were present within the segment encased, and in the other 2 before a general cavity was present, the former being thus the less developed. At this time also there was no mechanical tissue present.

The first plant was examined 20 days after the cast was applied and was one of the 2 in which certainly no dead cells were present in the pith at the time of application. This plant as well as the other 3 grew about as well as its normal neighbors. Elongation was not complete till after casting, and hence the swelling above the cast was gradual, not sudden.

Above and below the cast a large cavity was found in the pith, its size above the gypsum being two thirds the diameter of the stem. Within the cast was no cavity, but there were a few dead cells crushed together in the centre, closely pressed by the surrounding living tissue. The mass of living pith cells within the cast was greater than that above and below. The preexisting large intercellular spaces in pith and cortex had been nearly closed up. The bundles within and outside the cast were about equally well developed. Above and below the cast the bundle-sheaths were beginning to show thick walls; within the cast all cells were thin-walled. There was no evidence that the bundles had been crowded toward the centre; the great spaces in the cortex had afforded room for their growth.

The other 3 plants were examined after 52 days. They had all grown well and had produced ripe seeds.

In the one in which there was assumed to be no dead cells at the time the stem was surrounded with gypsum, the changes between normal and abnormal growth had gone on in the same direction as

indicated in the plant earlier examined. The pith within the cast had died nearly out to the bundles without collapsing; hence there was no cavity. The cavity above and below was normal. The intercellular spaces in the cortex had been in the abnormal segment completely closed up. The bundles had grown about as large within as outside the cast, but many vessels in the constricted part were flattened, not all in the same direction. The normal sclerenchyma sheaths of the bundles were well developed outside the casts; within, the thick-walled condition was entirely absent. There had apparently been little or no movement of bundles toward the centre.

The other 2 plants may have had small clefts in the pith when the gypsum was laid around them, but they certainly had no general cavity. The stems above the casts were not a great deal thicker than within. The central cavity was present through the parts in casts. The cortical intercellular spaces were not wholly closed. The bundle-sheaths within the constricted segments showed in each plant slightly thickened cell-membranes, but still much thinner than above and below these places. Because of the very loose tissue lying outside and inside the ring of bundles, the pressure here which had influenced to so large an extent the thickening of cell-walls could not have been very great.

One of the stems had increased in size outside the cast considerably more than the others, and at the upper limit of the cast showed the inner ends of the sclerenchyma sheaths stronger than anywhere else in the stem.

Lamium garganicum L.

In the stems of *Lamium garganicum* that have reached a height of several internodes above the soil, the cavity is usually found in the 4th internode from the apex, beginning about the time the cambium-zone is completed. The cavity arises from the union of several schizogenous clefts and enlarges lysigenously.

This plant is little suited to the kind of experiment here made; for out of 5 about the upper part of whose stems casts were placed, none grew well and only 2 reached a length of several centimeters above the gypsum, the other 3 dying after a period of several weeks, without growing.

The 2 plants which survived the treatment were examined, the first 24 days, the 2nd 45 days after the casts were applied. They had developed about equally however in these different intervals, and

gave exactly the same results. The pith in the upper internode within each cast was living, while it was dead but not collapsed in the lower internodes within the casts. Above and below each cast was a large central cavity. Except the addition of a few vessels in each bundle and the obliteration of intercellular spaces there had been no changes within the casts. Above and below the cast in each plant cortical cavities had appeared, the cambium-zone had been completed, and the cambium and the outer pith-cells had given rise to a few thick-walled elements. Within the casts no cells were thick-walled except the collenchyma at the angles of the stem, and this condition of this tissue was present before the casts were applied. The bundles had not grown so much within as without the casts; within, vessels were smaller and somewhat crumpled.

In both plants the mechanical tissue was better developed at the upper limit of the casts than within or farther above.

Cucurbita pepo L.

In the aerial parts of *Cucurbita pepo* the cavity is present everywhere before elongation has ended, and everywhere, except in the hypocotyl and the flower stalk, before enough surface of an internode has been exposed for the application of a cast. When however a cast is applied to a young hypocotyl not later than the unfolding of the cotyledons, or is applied to the flower-stalk, there is a cessation of growth lasting for a week or more. In 3 flower-stalks put into casts 2 of them soon died and the 3d in a period of 2 weeks developed no farther except to increase in thickness by the expansion of cells above and below the cast. Out of the large number of very young plants whose hypocotyls were put into casts, 10 grew but only a few grew well. The plant sometimes forms a thick swelling just above the cast and sometimes has only the normal thickness. If the cast is applied after the plant has produced several leaves, the disturbance of growth is much less.

The cavity appears first as a schizogenous split in the middle of the pith, the separation of cells being soon followed by their death. As the cavity enlarges the tangential pull on the cells bounding the cavity is plainly indicated by the distortion there evident. The cambium-zone is not completed for some time after the cavity appears.

1. *Examination after 28 days.* About the hypocotyl of this plant the cast was applied when the first leaf between the cotyledons was

beginning to unfold, probably then before a cavity was present. The plant in the 4 weeks grew to a length of 6 internodes showing a weak stem with small leaves. Above the cast was a small swelling. The stem had grown considerably in thickness above the cast, very little below.

Within the cast a small cavity was present, nearly filled by the pushing of cells. The cortical and pith cells and the bundles were elongated radially. Since the application of the cast, there had been some growth from the cambium in the bundles, and a few cortical cells at the outer end of each bundle began to show thick walls.

Outside the cast the increase of elements in the bundles was greater than within. The most of the increase in thickness of stem came however from the increase in size of cells. Hence all parenchyma cells within the cast were smaller than those without. The cortical sclerenchyma above the cast was much thicker-walled than within. Below the cast the thickness of wall was not greater than within, — an abnormal condition.

Another individual whose hypocotyl at a still earlier period of development than the preceding was put into a cast, grew for 38 days afterward, but then had a weak development. The external and internal changes caused by the cast were insignificant. A cavity was present within the part in the cast.

2. *Examination after 48 days.* Five plants when the first leaves were beginning to appear between the cotyledons had their hypocotyls encased in gypsum. The longitudinal growth of the stem in the 4 weeks varied between a length of $\frac{1}{3}$ and $\frac{2}{3}$ of a meter. Differences within and without the cast varying with the length of stem and leaf-formation were found. In no cases were large swellings present above the cast; 2 of the plants had grown nearly as well as normal ones in the same pot.

Within the cast all of the plants showed cavities with stretching of cortical and pith cells toward the centre. The weaker plants showed thick-walled cells in the sclerenchyma of the cortex only. The 2 stronger plants showed also thick-walled parenchyma cells among the vessels, but only in part of the bundles. The bundles showing these thick-walled cells were the largest and lay opposite broken-down tissue in the cortex. Where the cortex was intact, the bundles were smaller, weaker and distorted.

Above the casts the bundles were normal, showing more mechanical cells than within, except in those bundles within which lay opposite the destroyed cells in the cortex. Above the cast the cortical sclerenchyma was also stronger than within.

Below the casts the cortical sclerenchyma and the xylem parenchyma had both thinner walls than above the cast, and consequently thinner walls than normally.

3. Examination after 66 days.

a. Three plants similar to those just described were treated in the same way but allowed to grow for 66 days. One of them was less developed than the others and probably had no cavity in the hypocotyl when the cast was applied. This younger plant produced above the cast in this time 6 internodes with a total length of 10^{cm}. The other 2 plants had reached a length of nearly a meter. Cavity was present within and without the casts. A record of the histological differences in these plants would be a simple repetition of that for the foregoing 5 plants. One point however should be noted: 2 of these plants showed abnormal swellings above the cast, and sections showed that all kinds of cells had taken part in forming this greater thickness. Cortex, pith and cambium were here abnormally active in cell-division.

b. One very vigorous plant with 5 well developed leaves had its 2nd epicotyledonary internode encased. The plant grew better than any of the younger ones in casts, showed no abnormal swelling, filled the cavity within the constricted segment, and gave the same general results that the others gave. The preexisting cavity gave room for the formation of nearly normal bundles. All thin-walled cells remained smaller than above and below the cast, and thick-walled cells were fewer and weaker.

In part of the foregoing plants the pith was better preserved within the cast than without, but generally the plants made such poor growth that much could not be decided on this point.

Althaea tauriensis DC.

The cavity-formation in *Althaea* is first indicated by the appearance of large intercellular spaces among groups of cells in different parts of the pith. Such groups are separated both in cross and longitudinal sections by intervening tissue composed of more compact cells. The mucilage cells of the pith play no especial part in the breaking down of tissue. The large intercellular spaces run together to form clefts, and immediately the bounding cells collapse. Thus there arise in the pith many cavity-islands separated by vigorous looking cells containing a good amount of protoplasm. This condition which does not come into existence till after elongation has ceased

may continue for weeks. By the progressive death and collapse of cells the separate cavities unite to form a hollow pith.

In 5 young plants the 2nd internode above the soil was put into a cast before any cavity was present in the pith and before elongation had ended. The plants grew fairly well, and without distortion.

Two plants that had doubled in size in the interval were examined after a period of 18 days. The pith was intact throughout the plants. Above and below the casts the thick-walled cells in phloem and xylem were beginning to appear. Within the casts there had been less multiplication of cells from the cambium and no thickening of cell-walls.

The 3 other plants were examined after growing within the casts for 50 days. One had attained a height of 20^{cm}, one 35^{cm} and the other 60^{cm}. The smallest plant showed below the cast a large central cavity, above the cast several small cavities, and within the cast a solid living pith. Above and below the cast many mechanical cells showed in phloem and xylem, while in the cast only 3 or 4 bundles showed 3 or 4 thick-walled cells in each, some of these thick-walled cells being hard bast and some xylem parenchyma.

The plant of medium development had a large central cavity below the cast, and several small cavities above and in the cast. The mechanical tissue was within the cast much more strongly developed than in the preceding case, but still was of fewer and weaker cells than in the same stem above and below the cast. Room for the small widening of the bundle ring had been made by the compression of the pith.

The third plant and the one of the greatest development showed small, isolated cavities above and below the cast, but still a greater proportion of dead pith within the cast. The bundle ring here as in the preceding cases was at a much smaller distance from the centre than in normal parts of the stem. But in this individual the bundle ring had within the cast nearly as many cells in a radial row as in the same stem above and below the cast, averaging 40 outside the cast and 38 inside. The proportion of thick-walled cells was however less, but not greatly less, within the cast than without. This great increase in thickness of the vascular ring has taken place at the expense of the pith which is greatly compressed. Because of this movement of tissue toward the centre, the living pith cells and the elements of the bundle, especially those of the xylem are much elongated radially.

In none of these plants does the cortex either in the normal or in the abnormal parts of the stem have cavities. In none of them

are the cortical cells noticeably elongated in the radial direction except in those places where the elongation can be accounted for by growth outwardly, where the gypsum by a leaf base or other means was kept from confining the expansion.

In all these plants there was an excessive development of thick-walled cells in phloem and xylem at the upper limit of the casts.

Urtica dioica L.

In *Urtica dioica* the time and place of the first appearance of the cavity is very diverse. A young thick stem that is growing well will first show a cavity when a height of 6 or 7 internodes above the earth has been reached, the 3rd or 4th internode being the first to become hollow. A stem may attain a development of 12 internodes above the soil and still be completely solid. The cavity may appear either before or after elongation has ceased; it has never been found nearer than 3 or 4 internodes from the apex of the stem. These observations show that the cambium-zone may or may not be complete when the cavity shows itself.

Before a general cavity is present several schizogenous clefts are found in the pith which are isolated longitudinally and transversely. By the union of these clefts the general cavity arises and then enlarges schizogenously and lysigenously.

In the experiments on this plant 6 casts were applied around the 2nd and 3rd exposed internodes from the apex. Cavities certainly were not present in these internodes at the time, and the cambium-zone was not complete. The casts at first affected the growth of the plants but little. There were no swellings nor distortions; and had the succeeding weeks not been very dry the growth would probably have been much greater.

1. *Examination after 23 days.* This plant had grown nearly as well as the normal ones by its side, having added above the cast 4 internodes with a total length of 9^{cm}. The cavity had extended no farther within this period, the pith being solid from below the cast to the apex of the stem. Growth had taken place within the cast, since the cambium-zone was complete, and the ring of secondary tissue contained 6 radial elements. Except the collenchyma, which was present before the experiment began, no thick-walled cells were to be found within the limits of the gypsum. Within the same segment the preexisting cortical and pith intercellular spaces had been closed up.

Above and below the cast were splits in the pith and cavities in the cortex. The elements derived from the cambium ring numbered radially 2 more than within the constricted segment. Thick-walled cells had made their appearance in the usual places, both above and below the cast, — in the phloem, the xylem and in the cells limiting the xylem centrally. At the upper limit of the cast the thick-walled xylem parenchyma was greater in quantity than above or within the cast.

2. *Examination after 40 days.* Before this time 2 of the 5 plants in casts had died and the remaining 3 showed various degrees of development, none of them reaching more than 12^{cm} above the cast. All of them showed within the cast some changes not present in the plant examined after the interval of 23 days.

Each of these 3 plants had a central cavity below the cast, none within and only 2 of them a small cavity above.

All of them showed a solid living cortex of radially elongated cells within the cast, but cortical cavities above and below, with little destruction of tissue and cells with radial elongation, which however may have been caused by the room for growth afforded by the gypsum not filling all the unevennesses of the surface of the stem. All of them had within the cast a little mechanical tissue besides the collenchyma. The first thick-walled cells to appear in the constricted segments were the xylem parenchyma; in the stoutest stem bast fibres and lignified cells bounding the bundles internally were also present. Within the casts the thick-walled cells were in no case half so numerous as in the same stem above and below the cast. The cells which have become thick-walled since the last examination are evidently not cells that have been formed since that time. The vessels in all 3 stems in the constricted segments are crumpled and smaller than normal. The xylem parenchyma thick-walled cells are generally smaller within the casts than without, but still they are no smaller than the smallest outside the cast.

One of these plants, the one that shows the greatest growth, has at the upper limit of the cast more than the normal amount of thick-walled xylem parenchyma.

Dahlia variabilis W.

The cavity appears in the pith of *Dahlia* at various ages of the plant, the time of its beginning seeming to depend more on the radial than the longitudinal extension. In the average stem the pith

will be found hollow in all the internodes except the lowest or the 2 lowest long before primary growth has ended. It is not unusual however to find slender stems with 3 or 4 of their lower internodes fully elongated and solid; and one stem was found where the cavity showed first in the 7th internode above the soil, at least 5 lower internodes having completed their elongation. This would seem to be a clear case of normal preservation of the pith where the usual rapid, radial expansion was not present.

Cavity-formation is at first indicated in those internodes where it comes into existence before elongation is complete by the inter-cellular spaces growing at the middle of the pith into small clefts. The collapse of cells then follows immediately. In those internodes where the cavity first appears after secondary growth has begun, the cells collapse without being separated by more than the usual inter-cellular spaces at the angles. The two methods pass one into the other.

Dahlia grows well or ill when surrounded with a cast according to the age of the plant at the time of application. A young plant stops its growth to begin it again after a rest lasting from one to many weeks. One plant after the cast was applied grew slightly in thickness and height for a few days, and then for 15 weeks made no farther growth while a normal plant of same age and size growing in the same pot reached the height of nearly a meter. At the end of these 15 weeks the resting plant was placed where the sun could shine directly on it from morning to night when growth again started and proceeded normally. Above the upper limit of the cast there is always found a swelling thicker than the normal stem, in which all tissues show themselves increased, the outer covering being of cork.

Five stems that were surrounded with casts have been examined.

1. The first of the series had 3 internodes above the earth when the cast was applied, and the second internode was enclosed. In the 30 days following, the plant grew well in length adding 4 internodes, but the growth in diameter was only 21% of its former thickness. Above and below the cast the bundles have grown in number of elements, especially in the soft bast and in the thick-walled xylem parenchyma. Moreover the cambium ring has been completed and has given rise between the bundles to a band of thick-walled tissue 2 or 3 cells in radial width, while the outer pith-cells have become thick-walled.

Within the cast there have been fewer cells formed, the phloem has grown but a little in both hard and soft bast, the vessels have increased by 2 or 3 in a bundle and are much distorted, less than half the number of thick-walled xylem parenchyma cells are present

here as above and below the cast, the cambium ring is not completed, and the outer pith-cells are not nearly so thick-walled. There can be no question but that the corresponding cells that within the xylem in normal parts of the same stem have become thick-walled are here still in their early thin-walled condition.

In this plant there was no cavity below nor in the cast and but a very small one above.

2. Three plants that, when but 3 or 4 internodes in height, had casts placed around the highest exposed internode and part of the next lower one were allowed to grow afterward for 4 months. They had not become strong plants and had reached heights varying from 35^{cm} to 40^{cm}. The weakness of these individuals is to be in part accounted for from the fact that weak plants were selected for the experiment, since only such have a living pith when they are at a size suitable for application of casts. All these plants showed the stem within the cast from $\frac{2}{5}$ to $\frac{3}{5}$ the diameter above and below; all of them showed the abnormal swelling just above the cast.

In internal structure all the stems showed cavities above and below the casts but none within, and less multiplication of cells, and less mechanical tissue within the casts than above and below. One showed within the cast in each bundle a small group of hard bast and a few thick-walled xylem parenchyma cells. Between the bundles was a band of thin-walled cells derived from the cambium and containing in a radial row 5 cells. Above the cast the thick-walled cells in a corresponding position numbered from 18 to 20. Within the cast the parenchyma cells among the vessels were thin-walled; above and below they were thick-walled. It is quite possible that the most of the thick-walled cells within the cast in the phloem and xylem were present in this plant when the cast was applied; the cambium-zone and its derivatives were certainly then not present.

A second one of these 3 plants showed much less development within the cast and much less formation of secondary tissue outside the cast than the preceding. The differences however within and without the limits of the gypsum were the same in kind. In this case the plant must have been less differentiated internally than in the preceding when the cast was applied; but a few bundles in the constricted part of the stem showed any hard bast.

The 3rd plant had above the cast a secondary ring of thick-walled elements from 8 to 10 in a radial row. Within the cast the cambium ring had not been formed, and but for 2 or 3 thick-walled xylem parenchyma cells in each of 2 or 3 bundles, thick-walled cells were entirely wanting. Here was almost a complete suspension of

growth, only the meristem of the bundles having added a few small thin-walled elements.

It cannot be said that the thin-walled state of cells that normally would be thick-walled is to be accounted for alone by keeping such cells at a small size by confining the growth, since in all these 3 plants, these corresponding thin-walled elements are thick-walled in the upper 8^{mm} or 10^{mm} of the segment within the cast, though there, as below, the cells are no larger and the bundles no better developed.

3. One plant that was much stronger than the foregoing had a cast put about the 5th internode from the earth leaving one good-sized internode exposed above the gypsum. The plant grew as well as normal ones, reaching in 68 days a meter in height. At the end of this period its structure was examined.

Within the cast the preexisting cavity had been nearly filled by the growth of tissues toward the centre. All kinds of elements showed radial elongation. The hard bast was thinner-walled, and more thin-walled derivatives from the cambium were present than without the cast. The pith was better preserved within the cast.

A general condition for all these *Dahlia* plants should be added: The stem below the cast grows more slowly in thickness and produces much less secondary tissue than above.

Archangelica sativa Mill.

The pith dies first in the second internode above the soil, the cavity formation beginning schizogenously before elongation is complete, the separation of cells being very small however before their death ensues. Within casts this plant makes good growth, no abnormalities appearing outside the gypsum. Three plants had upper internodes put into casts, elongation not being complete in any of them, and probably no cavity existing.

Twenty days after the application of the casts all 3 plants were taken for examination. One had added 10^{cm} of new growth, the 2nd 15^{cm} and the 3rd nearly 20^{cm}, these differences corresponding to the different strengths of the plants 20 days before. All showed a marked constriction where the gypsum had surrounded them.

In the plant of the weakest growth there was a very small cavity present within the cast, one of about equal size above the cast and a much larger one below the cast. Soon after the cast was removed however the cavity within grew larger by the expansion of the compressed stem. It is even doubtful whether a cavity had really existed

within this constricted part before the removal of the cast. But the question is not of importance since the fact remains that the cast had preserved more pith than would have lived normally. The changes which by comparison with normal plants can be seen to have taken place within the cast are the following: The bundles have grown in number of elements, but not appreciably in size. The thick-walled vessels present when the cast was applied averaged not more than 6 to a bundle; they are now double that number and distorted, the flattening having no regular direction, being now in the radial direction, now in the tangential and again in an oblique direction. A few vessels which have remained thin-walled have been added and they have not been flattened. The phloem has also increased in number of cells but is also distorted. The meristem between the bundles shows no appreciable change except in a slight radial elongation of cells. The cortex shows no change except an appreciable thickening of the membranes of the collenchyma groups. Thus in the bundles where there has been evident change, the room for growth has been mostly if not altogether made within the bundles themselves by the flattening of elements. The minute size of the intercellular spaces present in this stage of the normal plant has not here afforded room for growth that many other plants have shown.

Above the cast at a distance of 15^{mm} or less the stem shows normal structure. The number of elements in the bundles is no greater than in the part within the cast (normally of course the lower the section is taken the more elements we should find in bundles), but there is no distortion, and more vessels and phloem cells have had their walls thickened. Moreover the cells bounding each bundle inwardly and those bridging the interfascicular spaces show thickening walls. The collenchyma in the cortex has much thicker membranes than within the cast.

Below the cast the differences noted between the 2 preceding cases are much more strongly emphasized. There the secondary formation has proceeded for some time and the mechanical tissue is strongly developed in and between the bundles and in the cortex.

In the plant of middle growth the pith within the cast was all living, while above and below were good sized cavities. All of the differences noted in the preceding case between the normal and the constricted portions were more strongly marked in this. Either this stem when encased was less developed internally than in the preceding plant, or the small cavity found within the cast in that plant had afforded room for growth; for in this plant the bundles had fewer elements within the cast than in the corresponding position in the preceding.

In the upper part of the portion within the cast in the plant of greatest growth there was a minute spot in the middle of the cross-section filled with a few fragments of cell-walls. The conditions existing within the abnormal parts in the 2 preceding cases existed here also. The only difference noted was that there had been in this 3rd plant more elements formed within the bundles than in the other two. But all of these elements here as in the other cases had remained thin-walled. In a normal plant of a development such as this one had at the time of application of the cast and sectioned in like position, the number of thick-walled vessels in an average bundle was 8 to 10; the number of vessels whose walls had not become thick 15 to 18. Within the cast the vessels of like description numbered thick-walled 12, thin-walled 28 to 30. Above the cast in the same plant there were in an average bundle 40 thick-walled vessels and 12 thin-walled. Though the number of vessels thus seem in the abnormal part to be about $\frac{4}{5}$ that in the normal part of the same stem above the cast, the diameters of the stem at the 2 positions are as 5 to 9. The cross-section of an average bundle within the cast is also, proportionally to that of such a bundle above the cast, very much less than the number of vessels would seem to indicate; for within the cast all bundle-elements are much smaller than their normal size, and all bundles lack a wide band of mechanical xylem which is present in the normal parts of the stem just centrally from the cambium.

Thus there are presented in this stem, in 2 positions within 15^{mm} of one another, one section with mechanical tissue represented only by the very weak collenchyma in the cortex, and the other section in which the collenchyma is very strong, the hard bast well developed, the xylem well provided with mechanical elements and a bridge 6 or 7 cells deep, with strong walls, extending through the outer part of the pith from bundle to bundle.

In the lower part of this 3rd cast, the portion of the stem there enclosed belonged to an internode below that last described, and hence was much thicker. The cavity there present when the gypsum was laid around it had since been filled by the inward growth of all tissues, all showing cells much radially elongated, and a little mechanical tissue had appeared in the cortex, in the phloem and on the inner border of the xylem bundles. The mechanical tissue a little below this position and hence outside the cast was almost incomparably stronger.

In all these plants the stem at the upper limit of the cast showed more thick-walled tissue than normal especially in the outer part of

the pith. All plants showed within the limits of the casts smaller pith-cells and cortical cells than normal.

Myrrhis odorata Scop.

In structure of stem and formation of cavity, *Myrrhis odorata* is very similar to *Archangelica sativa*.

Four stems none supposed to be hollow had each an upper internode surrounded with gypsum. At the end of 27 days one plant had died, and the others not looking well were cut for examination. In none of them had the stem above the cast grown greatly in thickness. All of them showed the central cavity above and below the cast, and one had so large a cavity within the cast that it is probable that it was there when the cast was applied. A second plant showed within the cast a very narrow but long seam in the middle of the pith, composed of a few cell-remnants crowded together by the surrounding living cells. It is probable that the dead cells in this pith had died after the gypsum was laid around the stem. The 3rd plant showed no cavity within the cast, but a central cylinder of some size in the pith was composed wholly of dead, uncollapsed cells. The outer ring of dead cells had been somewhat crumpled by the pressure of the slightly centrally extending surrounding tissue. To one acquainted with the behaviour of the plant, there can be no doubt that the core of cells died after the cast was applied. In all 3 of these plants however the cast was markedly influential in prolonging the life of the pith-cells, since everywhere within the casts there were many more rows of living pith-cells between the bundle-ring and the dead cells at the centre than either above or below the casts. It may be added also that in all cases the living pith within the casts was composed of smaller cells than the living pith beyond the limits of the cast.

In regard to the conditions of the other tissues within, at the limits and without the casts, it may be said briefly that the same relations existed here in all 3 cases as have been narrated for *Archangelica sativa*. A description for *Myrrhis* would be merely a repetition of the one given for that plant.

Vicia faba L.

The first appearance of the cavity in *Vicia faba* is in the 3rd epicotyledonary internode when the plant is but 3 or 4 internodes in height. From the 3rd internode the cavity-formation progresses upward and downward, being always found in the internodes near the apex before they have elongated, but not appearing in the hypocotyl till the plant is more than half grown. Thus the cavity is not present in the hypocotyl till long after elongation has ended, appears in the 2nd epicotyledonary internode when elongation is nearly complete, and in all the other internodes long before extension ceases.

The phenomena of cavity-formation during primary growth are these: The pith first shows ordinary intercellular spaces which rapidly enlarge giving a central spongy parenchyma. The intercellular spaces run together forming in cross-section several large openings separated by chains of cells. As these schizogenous spaces still farther enlarge, several cells are left with but slight connection with the neighboring tissue, and these cells are the first to die. The several spaces a little later, both schizogenously and lysigenously, unite to form one cavity which then constantly enlarges by the extension of the outer rows of pith-cells and the stretching and eventual death of the inner rows of pith-cells, the bundle-ring meanwhile moving farther and farther from the centre.

Etiolation does not prevent the formation of cavity; it produces merely a narrower cavity. Four plants were grown in darkness with the result that the cavity-formation appeared in the usual way in each. One plant had a wrapping of black cloth placed about the 3rd internode and so grew for 46 days. Examination at the end of this period failed to show structural differences induced by the opaque wrapping. Two plants however that had casts about the 3rd internodes and also grew in darkness showed the same differences within the casts as those grown in light and later to be described. Etiolation here as generally brings a weaker development of mechanical tissue, but causes the disappearance of no one kind of mechanical tissue.

Experiments with Gypsum Casts.

In the experiments on *Vicia faba*, casts were applied to more than 50 plants, and about an equal number of control plants have been used. *Faba* suffers very little from the cast, plants in casts

usually growing as well as normal ones by their side; nearly always the effect of the cast on the cellular structure extends less than 5^{mm} below and 10^{mm} above its limits. Still it can be said that there is a tendency in such plants to the production of a slightly weaker individual. Since the most of the growth in thickness takes place in this plant before elongation is complete, casts must be applied before elongation is complete, and as a result the swelling out of the stem above the cast is gradual, not sudden. The experiments fall into 2 classes: those in which the casts were applied before the cavity in the stem was present, and those applied after the appearance of the cavity.

With Casts applied before the Presence of a Cavity.

For this series of experiments 7 plants were put into casts, the plants exposing at the time but 3 internodes, and the cast enclosing the 3rd internode and all or a part of the 2nd. Plants similar to those encased in gypsum were preserved in alcohol for future comparison.

1. *16 days after casting.* A study of control-plants of like age and development with the one from the cast shows that the normal changes in these 16 days have consisted in the enlargement of cells generally, in the formation of greater intercellular spaces, in the addition of many vessels to each bundle, and in the formation of a central cavity in all internodes above the epicotyl. The cambium-ring has at this period not been completed and no mechanical cells have appeared in the bundles.

These normal changes are present in the plant from the cast, above and below the limits of the gypsum. Within the cast the cells have become but slightly larger, the intercellular spaces smaller, and the vessels more numerous than 16 days earlier. The vessels within the cast are smaller, crumpled and number about 8 in an average bundle. Above the cast they are larger, of normal size and number about 20 to a bundle. The cast included the 2nd epicotyledonary internode and hence there is no cavity below. Within is no cavity; above is a cavity about $\frac{1}{4}$ the diameter of the stem. Within, the cells of the cortex, especially in the outer rows are elongated radially, and as everywhere else in the stem the cambium between the bundles has not formed.

2. *33 days after application of cast.* The normal progress of growth up to this time showed these features, in the 2nd and 3rd internodes: The cambium-ring had been completed, and had given

rise to a narrow band of secondary tissue. The bundles had increased greatly in size, the vessels in an average bundle now numbering 40 or 50, while in each group of phloem were 10 to 20 thick-walled bast cells where none had existed 33 days before. In the normal stem and also in the one in gypsum above the cast was a cavity in the pith more than $\frac{1}{3}$ the width of the stalk; the remaining pith-cells had enlarged and those lying next the bundle-ring had become thick-walled and lignified. Below the cast, this being in the epicotyl, there was no cavity and no thick-walled pith-cells, — a perfectly normal condition.

Section taken through that part of the 3rd internode that was within the cast, should be compared with the sections described as taken above the cast rather than with those below. Observations on the structure of the encased part of the 3rd internode teach that little development has taken place within 33 days. The cortex shows its cells with smaller intercellular spaces than before the cast was applied, and with greater radial elongation. Cambium is nowhere to be seen between the bundles. In the phloem are no thick-walled cells. In the xylem, instead of 2 or 3 lignified elements in a bundle as 33 days ago, are 40 or 50, — partly vessels and partly wood-parenchyma. To form this group of lignified elements the weak cells there present 33 days ago have grown but a little in size. The cells have apparently enlarged, become somewhat thick-walled and lignified. These elements are, it is to be noted, of smaller size than they occur in normal growth. The pith is solid and all its cells are living. The only change that has taken place here since the cast was applied is in the closing of the intercellular spaces.

At the upper limit of the cast there is an excessive development of mechanical tissue in the xylem and in the outer part of the pith. Above the surface of the cast where the stem enlarges from the constriction there is more of this thick-walled tissue than anywhere else in the stem. Below this surface and hence within the constriction, the amount of such tissue is less, but still much more than well within the cast. Cells with the same relative position and the same size as those with very thin walls 1^{cm} below the surface of the cast, have walls increasing in thickness as the upper limit of the cast is approached.

3. *43 days after application of cast.* Above and below the cast the normal development had gone on in the tissues. The large intercellular spaces in the cortex had grown to tangential splits extending the length of many cells, the spaces being bounded by

collapsed elements. More mechanical tissue had developed in both phloem and xylem, and the cells of the fundamental tissue bounding the bundle-ring toward the centre had become thick-walled. Above the cast was a good-sized cavity in the pith, and below the cast was one the diameter of a single cell.

Within the cast, in the cross-section studied, in two places just beneath the first hypodermal row of cells there had been for the length of several cells a slight crushing of tissue, the space thus afforded having been completely closed up again. In cambium-formation there has been a little progress from the last plant described, since the primary cells between the bundles have become elongated and in most place chambered, though nowhere has secondary tissue arisen between the bundles. In the phloem the cells which normally become thick-walled have increased in size in cross-section, but they have not attained the size of the normal thick-walled cells and none of them have become thick-walled. In the xylem the number of vessels has slightly increased in each bundle. All of the primary vessels and all of the earlier secondary vessels have been completely flattened together, while the vessels more recently formed are of the usual shape, but of smaller size. Among these recently formed vessels are a few thick-walled xylem parenchyma cells. In the pith there is no cavity and the cells are living. At the sides of each bundle a few cells of the fundamental tissue have become thick-walled and lignified. In these 2 places where mechanical tissue has made its appearance, — in the xylem and flanking the xylem bundles, the mass is extremely small when compared with the amount in similar positions in the normal structure.

In this preparation, as in the preceding, the great increase in mechanical cells at the upper limit of the cast is noted.

4. *55 days after application of cast.* Two plants of equal age and like treatment were examined. Both of them were well supplied with blossoms and had reached a height of about a meter, though one of them was not so strong a plant as the other. In both, the tissues above and below the casts were well developed, and well supplied with thick-walled, supporting cells. The weaker plant had no cavity below the cast — this was normal since it was in the 1st epicotyledonary internode — but had a large cavity above the cast; the stronger plant had a cavity below the cast — in the 2nd epicotyledonary internode — and also one above. There were no cortical cavities, but the tissue was very loose.

The weaker plant showed within the cast the same condition of tissues as noted in the plant 43 days in gypsum, except that within

the cortex there had been no collapse of cells, and that the cortical cells instead of as before being longer radially, were longer tangentially and had wavy membranes.

The stronger plant shows a stage more advanced in development, in that the interfascicular cambium has given rise to one or two rows of cells, and more mechanical cells are present within the xylem and in the fundamental tissue limiting the bundle-ring toward the centre. In the phloem a few cells show membranes slightly thickened. In the cortex a few cells have collapsed in 2 places, the spaces being again completely filled. In other respects these 2 plants in the part within the casts show all the features of the one described at the period of 43 days. In both of them the vessels and xylem parenchyma cells are narrower and thinner-walled than outside the cast, and the corresponding cells that toward the middle of the cast have remained thin-walled have nearer the upper limit of the cast become very thick-walled, though the cell-diameters have not increased.

5. *59 days after application of casts.* Two plants gave upon examination the same results as the 2 last plants described. Within the casts were no cavities either in pith or cortex, and no thick-walled phloem cells. The cell-walls of both pith and cortex were wavy. In the one plant where the thick-walled cells of the xylem are very few within the cast the cortical cells are radially elongated; in the other plant, though the cortical cells evidently were earlier radially elongated, they have by growth of the bundle-ring been so crowded and wrinkled that they now appear with the tangential diameter the greater.

The usual increase of mechanical tissue at the upper limit of the cast was present in these 2 plants.

With Casts applied after the Presence of a Cavity.

In this series of experiments casts were put around 18 plants that had grown to 4 internodes above the soil, the 2nd, 3rd or 4th epicotyledonary internode being encased. The first examination was made after the cast had been on 13 days, the last after an application of 80 days. The longest interval between examinations was from the 36th to the 54th day. All the other intervals were ten days or less. The oldest plants were 3 feet or more in height and had passed, by several weeks, the blossoming time.

Without exception this series of plants gave the same general results that the first series did: Within the cast all tissues developed more slowly than without, but developed continuously; the mechanical

cells were especially slow in becoming thick-walled, the hard bast appearing last of all; the cortex was always better preserved within the cast than above or below, as was the pith.

The changes found in this series and not occurring in the former were those called forth by the presence of the cavity. The earliest change within the cast is the enlargement of the cortical cells; and since they cannot grow tangentially, they stretch out radially forcing all cells which lie centrally from them inward and thus causing all other cells to elongate radially. In this movement of cells the pre-existing cavity is partly or wholly filled by the living pith-cells which crush the remains of collapsed cells together in the centre. While this movement of cells is taking place toward the centre the cambium begins the formation of secondary tissue, more tardily than normally, but earlier than in stems without cavity and in casts. The resulting mechanical cells of the bundles are somewhat smaller than in the normal stem and less numerous, but more numerous than in the stems of the 1st series of plants.

Melianthus major L.

A young shoot of *Melianthus major* growing from the earth will remain without cavity till it has reached a height of 4 to 6 internodes, the 2nd or 3rd internode being the first to become hollow. When the cavity is once formed however it spreads rapidly upward extending into the internodes still enclosed in the sheathing bases of the terminal leaves. There is thus shown a great change in the relation of the time of cavity-formation to the termination of elongation. When the shoot growing from the earth is but a few internodes in height elongation has ended with the pith still solid in the lower 2 or 3 internodes; when the shoot has attained a greater height the cavity is present long before elongation is complete, usually before the internode has attained $\frac{1}{10}$ of its length.

The beginning of cavity-formation is the separation of cells by the growth of intercellular spaces in the middle of the pith. There the cells of the central mass have more surface exposed to the intercellular spaces than is in contact with neighboring cells. Soon after a cell has all of one side thus exposed it dies and collapses. Nowhere could dead cells be found that were not thus exposed, while small rents could be seen to be bounded by living cells.

In the research on this plant 7 individuals had internodes enclosed in plaster casts and 2 in opaque envelopes. The opaque wrappings

were 6^{cm} in length, remaining about the stems for 5 or 6 weeks, but produced no decided changes internal or external. Of the 7 stems put into gypsum casts, 2 had then but 3 internodes above the earth and therefore were not hollow, while the other 5 were older shoots and were certainly hollow when the casts were applied. Since the increase in thickness in the stem is extremely slow in the older internodes, the casts were in all cases put around the highest exposed internode or the 2 highest. The two shoots about which the casts were placed supposedly before the cavity was present, showed cavity within the part in the cast when examined some weeks afterward. All 7 plants may then be considered as forming one series of experiments. None of them seemed to suffer in any way from the effect of the cast.

Instead of considering the different individuals according to the length of the period under which they were under experiment, it will be better to take them up in the order of their development subsequent to the application of the casts, since the amount of growth is not graded to the length of time.

1. The plant which on examination showed the least development was in the cast for 48 days, in that period having added but 2 internodes. The position of the cast was marked by a decided constriction. A general movement of cells toward the centre had taken place, since pith, bundle and cortical elements were radially elongated. Pith and cortical cells were smaller than outside the cast. The number of secondary elements in a bundle below the cast was greater than that within, and greater within the cast than above, indicating that the pressure had not been sufficient to reduce noticeably the multiplication of cells. The increase in thickness of cell-wall has been decidedly affected. Below the cast is a band of thick-walled secondary xylem cells averaging 8 in a radial row, and outer pith-cells are thick-walled and lignified. Above the cast a zone of thick-walled pith, 2 cells in width, is shown as well as a group of thick-walled cells capping the inner end of each primary bundle. Within the cast are only thin-walled cells. In the last position the mechanical tissue would normally be considerably in excess of that above the cast. The number of cells in a radial row in the pith number above and below the cast 24, within 28.

2. This shoot being next to the foregoing in point of development, was one of those having the cast applied when there were but 3 exposed internodes above the earth. It has grown well and added 3 internodes. The cavity found within the cast was very small, — the living pith pressing a few cell-remains together in the

centre. The radial elongation was not so great. The number of cells arising from the cambium has been reduced by the pressure of the cast; since below the constriction the secondary xylem elements number 26, within 16 and above 14, whereas the proportion would normally be about 26:20:14. The prevention of growth in the thickness of cell-wall is marked. Within the cast the zone of thin-walled secondary elements lying centrally from the cambium is 2 cells greater in width than outside the cast. The number of thick-walled secondary xylem cells in a radial row is below the cast 24, within 12, above 12, — the intermediate segment not showing as it would normally an intermediate number of such cells. Moreover within the cast all of these xylem cells are considerably thinner-walled than above and below. Apparently the cast has affected no change in the number of phloem cells relative to the xylem cells. Above and below the cast is a wide zone of outer pith-cells thick-walled and lignified. Within, these cells have preserved their weak condition.

A new tissue has appeared in this shoot, — the phellogen lying just externally to the starch-sheath. Above and below the cast this zone is from 3 to 5 cells in width, and the cortex is beginning to break down. Within the cast the phellogen has not completed the circuit of the stem and the cortical cells are all intact.

3. The remaining 5 shoots have produced since the casts were applied respectively 3, 4, 5, 8 and 10 internodes in periods extending from 89 to 187 days. All of them show cavity throughout, but the pith is always better preserved within the cast than outside of it. All of them show radial elongation of pith-cells and of the oldest xylem cells. *In all, the number of mechanical cells in xylem and pith is within the cast in excess of that above and below the cast.* Inside and outside the limits of the casts the phellogen is everywhere present and the cortex outside of it is dead and shriveled. The secondary phloem has shared with the xylem this increase of cells within the casts.

Normally the size of the cross-section of thick-walled xylem cells varies greatly, the first formed being usually the larger. The size of these cells within the cast showed no important variation from the normal. The xylem cells that were formed before the cortex died have not attained their normal thickness of wall. They can be distinguished from those later formed by their radial elongation and thinness of wall. The total amount of mechanical tissue within the cast becomes greater than above and below, especially in the xylem. The 2 shoots that had grown the most after application of the casts showed the one over 40%, the other over 50% more tracheids within

than above or below the cast. The measurements on which this estimate is made were taken with an ocular micrometer on an imaginary line passing through the middle of the zone of xylem. Only complete cross-sections were used for this purpose. The irregular course of the zone in the part of the stem within the cast renders a computation by comparison of radii impossible. The only other factor needed is the average number of the xylem-cells in a radial row, since the size of the cells is about the same within and without the cast. These 2 cases thus measured are not exceptional, as the eye can easily detect the difference in amount of xylem in the normal and abnormal parts. The differences obtained are so large as to exclude the possibility of changing the general result through a more accurate computation.

The relations between the tissues within and without the casts can be conveniently expressed for the pith and the mechanical xylem in the following table:

Internodes added after the application of cast	Living pith-cells in a radius			No thick-walled xylem-cells in a radius		
	Below the cast	Within the cast	Above the cast	Below the cast	Within the cast	Above the cast
1. 3 internodes added	14	16	13	10	10	8
2. 4 internodes added	15	16	14	12	14	10
3. 5 internodes added	18	27	18	15	30	15
4. 8 internodes added	12	18	12	20	29	20
5. 10 internodes added	25	30	25	25	45	25

Ricinus communis L.

In this plant throughout the stem except in the hypocotyl the cavity is formed before elongation is complete. In the hypocotyl however the cavity appears first in the highest part after elongation ceases and progresses very slowly downward. One might expect therefore in these 2 parts of the plant to find the pith-cells concerned in cavity-formation behaving differently. But the early stages of this process are really very similar in the place where the ring of bundles is rapidly moving from the centre and where such movement has ended. In both places the pith before the cavity appears has intercellular spaces of moderate size filled with air. In the parts of the

plant where secondary growth has not begun and where elongation is still progressing, the intercellular spaces before any cells die grow so large as to divide the wall completely, but the space thus formed has never been found wider than the diameter of a cell or two before the collapse of surrounding cells begins. In the hypocotyl the cells collapse before any intercellular spaces have grown into clefts. In neither hypocotyl nor the parts above have dead uncollapsed cells been found. Thus, from the nature of the case, the cavity in the hypocotyl is formed only from the collapse of cells, while in other parts of the plant it is formed largely from the movement of the outer living pith-cells from the centre during the normal expansion of the stem.

Ricinus communis for months suffers very little from the application of casts. The constriction may be as small as $\frac{1}{2}$ the diameter of the stem above and below, but the plant seems to develop to the height of a foot or more just about as well as normal ones. How early this very narrow piece of the stem within the cast begins to show its effect on the development of the whole plant has not been determined.

The irritation given by the cast shows itself however at the upper and lower surfaces of the gypsum-cylinder. Above the cast the stem swells a little abnormally and gives rise to several lateral roots. Sections through this part show all tissues very active in cell-division. Below the cast the same effect is produced only much weaker in degree. For a centimeter or two above the cast the formation of cavity may not occur, and the whole segment of stem below the cast does not enlarge so rapidly as normally.

Two series of experiments have been made with *Ricinus*, one with lateral shoots from the stem and the other with young stems grown from seed.

1. For the first series the newly-formed branches on stems of one season's growth that had been in the autumn cut back and placed in pots in the plant-house were used. These branches often grew to a length of 3 or 4 inches including 5 or 6 internodes before any cavity appeared and hence were favorable for the study of that kind of cavity-formation which appears before elongation has ceased. More than a dozen of these branches were surrounded with gypsum, but they as well as the normal ones made but poor growth, and many of them died because the main stems bearing them did. Four grew sufficiently to give some results, adding after the casts were applied from 2 to 5 internodes. These branches all showed a better preservation of the pith within than outside the casts, and 3 of them showed

an entirely living pith within, but a hollow one above and below. Within the cast also the cells had remained smaller and had not multiplied to such an extent as above and below. The cortical cells were elongated radially and the vessels were distorted. There were no thick-walled cells within the casts, but all of the branches showed mechanical tissue below and 2 of them above the casts. Such mechanical cells were the hard bast and the xylem parenchyma.

2. In the second series 6 young plants had parts of the stem enclosed in gypsum, — 5 of them receiving the casts around the middle of the hypocotyl before the cavity had appeared, and one around the epicotyl after the cavity was there present.

The first plant in this series was taken for examination 30 days after application of the cast, the cast being about 3^{cm} in length and occupying the middle of the hypocotyl. The diameters of the stem below, within and above the cast were respectively 13 : 9 : 12. The diameters of the pith in the 3 positions were nearly equal, that below the cast being a very little greater than within, and that within being a little greater than above. The width of the cortex made more than half the difference in the diameters given, and next to it in amount of variation stood the bundle-ring. The cambium-zone was present throughout the hypocotyl before the latter was encased. During the 30 days, the secondary tissue that had arisen from the cambium within the cast did not exceed 2 or 3 cells in the radial direction. The changes that had taken place within the cast consisted almost solely in the addition of these few secondary cells and the thickening of walls of vessels, xylem-parenchyma and hard bast that existed in the thin-walled condition and in about the same size of cells before the cast was applied. These elements of the bundle-ring are fewer, a little thinner-walled and the xylem elements a little shorter radially than above and below the cast. The room for this very slight growth within the cast has been made by filling the intercellular spaces of pith and cortex. The number of secondary elements including the cambium that can be counted in a radial row are below the cast 12, within 7, and above 11.

The other 4 plants that received casts about the hypocotyl showed within the abnormal segments, which were from 3^{cm} to 4^{cm} in length, the same relations. They had grown after the application of the casts for periods varying from 65 to 100 days. The diameter of the stems in the normal part was from $\frac{3}{2}$ to 2 times that of the part within the cast.

In each case the cavity extended downward in the hypocotyl to within a few millimeters of the cast, and below that point was

nowhere present. In each stem cortical cells had died within and without the cast, and within the cast the more vigorous vascular zone had crowded the weak cortical cells so that a collapsed band coursed through the middle of the cortex. Without the cast the cortex, made very loose by the death of some cells and by the great intercellular spaces, retained its extended form. Thus within the cast the growing cells of the vascular ring made room for themselves by crowding together the weak cortical cells.

The pith inside and outside the casts had the same approximate diameter, the casts having been applied after primary extension had ended, and the power of growth not being sufficient to force inward the pith and the few secondary mechanical elements that may have been formed before the effect of the cast had made itself felt.

The effect of the pressure in retarding multiplication of cells was marked, since within the casts, the number of xylem elements derived from the cambium averaged in the 4 plants $\frac{1}{2}$ the number above and below the casts in the same stems. Thus within the casts the secondary elements numbered in a radial row in the various stems from 18 to 33, outside the casts from 30 to 65. The number of phloem cells derived from the cambium was proportionally reduced by the cast.

The xylem parenchyma cells showed much unevenness in thickness of wall, and were generally thinner-walled within than without the casts. They had a shorter radial diameter than usual, making them wider tangentially than radially, the normal relations of these 2 diameters being reversed. The vessels suffered the same shortening of the radial width. No change in the relative number of vessels could be detected.

The elements of the hard bast were everywhere fewer in number and provided with thinner walls than in normal positions.

It is worth noting too that though the cortical cells within the cast have been able to reach not much greater than half their normal size, the cells of the bundle show a proportionally much greater size.

In the plant about whose epicotyl a cast was placed before primary extension had ended and where a cavity was already present, the only point worth noting that differs from what has been yielded by the foregoing plants is the movement of all kinds of cells toward the centre, pith, fibrovascular and cortical elements showing radial elongation.

3. Envelopes of black cloth have been wrapped around lateral branches like those experimented with in the 1st series and around stems of young plants like those of the 2nd series, and though perfect etiolation has been secured none of the marked changes ascribed to the effect of casts have been found.

III. DISCUSSION.

1. Effect of gypsum casts on the functional activity of meristematic tissue.

Without exception the plants that have been under experiment have shown, when a cast has been put around the stem near the apex, a resting period lasting from a few days to many weeks. Some plants will rest much longer than others, but even in the same species the period varies greatly in duration. *Vicia faba* rests but a few days; *Cucurbita pepo* from a few days to a few weeks; two plants of *Helianthus tuberosus* have shown no growth anywhere, the one for 8 weeks, the other for 10 weeks, but are still living; while *Dahlia variabilis*, which rests usually for a week or more, gives one example where there has been cessation of growth for 15 weeks, but in which active development recommenced on exposing the plant to the full day's direct sunlight. In the two cases last mentioned, control plants in the same pots made very good growth, the Dahlia control plant reaching the height of nearly a meter, while the one in the cast remained at the height of 15^{cm}, though both were the same size and age when the cast was applied.

The case of *Juncus effusus* and *Allium cepa* was somewhat different where the whole growing shoot was encased in gypsum. The results are comparable with those obtained by Pfeffer in a work not yet published, but some pages from the manuscript of which he kindly permitted the author to read, where Spirogyra and the growing root-tips of several plants after lying for weeks in gypsum casts grew farther on being removed from the casts.

Many young shoots, 2^{cm} or 3^{cm} in length, of *Juncus* were wholly encased in gypsum, and examined after different periods of time, the longest period of confinement being 11 weeks. In all cases the young shoots were still living, showing the peripheral band of living cells wider than usual, but otherwise no change during their enforced cessation of growth. Whether such shoots would grow after release from the cast was not determined experimentally; yet from the behaviour of other plants under similar treatment there can be but little doubt that the growth would be resumed and would progress normally.

The 3 germinating bulbs of *Allium cepa* were also so encased

as to prevent growth except in the roots. (See page 4.) In these cases the bulb remained turgid and growth was almost at a standstill, while bulbs in normal relations had in the same period fully collapsed and mature leaves had been formed.

Thus in *Juncus* for 11 weeks and in *Allium* for over 4 weeks where extension of organs was made impossible by external resistance and all the other normal conditions of growth were present the meristematic tissue had retained its functional capability.

Several plants of *Vicia faba* by the use of casts have had the formation of interfascicular cambium in the 2nd and 3rd epicotyledonary internodes delayed for weeks after it has been formed above and below this position. In 2 such cases the casts were removed without injury to the plant, and within 2 weeks the weak abnormal segments had so grown in thickness as nearly to obliterate the external constriction left by the cast. Examination of the internal structure showed that the cambium-zone had been formed and had given rise to secondary elements in the usual way.

That the cambium itself may be preserved in its functional capability without being allowed to grow may be inferred from the results of de Vries¹ and Krabbè², the former stating that the multiplication of cells from the cambium proceeds more slowly under pressure, and the latter finding the size and thickness of wall of cambium-cells uninfluenced by pressure, till the pressure becomes so great as to cause all division to cease.

Three species of plants that have been under experiment have confirmed this point. Casts were applied to internodes of *Vicia faba*, *Dahlia variabilis* and *Ricinus communis* subsequently to the completion of the cambium-zone, and the plants so allowed to grow for periods varying from 5 to 6 weeks. Examinations of such plants show that within the given period the cambium produces a ring but 3 or 4 cells wide and that this increase takes place soon after the application of the cast. As soon as the intercellular spaces are filled growth is in these plants for the time given practically at a standstill. On the removal of the casts the cambium resumed its activity and within a few weeks produced as many cells as had been produced in the normal parts of the stem above and below the casts.

¹ De Vries, De l'influence de la pression du liber sur la structure des couches ligneuses annuelles. Extrait des Archives Néerlandaises, T. XI, 1876.

² Krabbè, Ueber das Wachsthum des Verdickungsringes und der jungen Holz-zellen. Berlin 1884.

In the 3 plants mentioned and in many plants with secondary growth the activity of the cambium cannot long be wholly held in check since, as will be shown later, room for growth will be made by the collapse of cortical cells. When however the space afforded by the collapse of the cortical cells has been filled cessation of growth must again ensue. How long meristematic cells would live while their division was prevented by their inability to extend themselves there is at present no means of knowing.

An interesting point noted by Krabbe¹ is that the cambium cells in the trees with which he experimented never divided under pressure till they had reached the size at which they divided normally. Pfeffer in his researches (not yet published) on the growing root-tips found that cells of the primary meristem divided within casts only at the same size as normal, while at a little distance from the primary meristem cells divided at somewhat less than the usual size.

Measurements to determine these relations in the plants here described have not been made, because the displacement and distortion of tissues render such measurements difficult.

2. Effect of gypsum casts on the growth of thin-walled parenchyma.

The first effect of surrounding with gypsum a stem in which primary growth has not ended is the filling, by the growth of the parenchyma cells, of the preexisting intercellular spaces. In this early change a displacement of tissues toward the centre may or may not take place². It was always present in the *Vicia* plants as also in *Melianthus* and in *Cucurbita*; in *Dahlia*, *Caltha* and *Althaea* it was not found; while in *Urtica*, *Archangelica*, *Myrrhis* and *Ricinus*, it was present in some cases, absent in others. The explanation of this difference in behaviour lies in the condition of pith and cortex at the time the gypsum is applied. If the pith has large intercellular spaces the bundles are very likely to be crowded toward the centre; for the cells of the pith in the plants under experiment enlarge less rapidly and vigorously than those of the cortex, and hence the cortex not only fills the intercellular spaces in itself but closes up those in the pith by crowding the cells there more closely together. In such

¹ Krabbe l. c.

² *Aristolochia siphon*, as described in De Bary's *Vergleichende Anatomie* p. 549, furnishes an example of compression of the pith as the result of normal growth.

plants as *Dahlia* and *Althaea* the intercellular spaces in the pith at the time the casts were applied were very small and the cells still vigorous, and consequently the movement toward the centre was not to be distinguished. In the 3rd group of plants noted, the movement toward the centre takes place or not according to the inactive or active condition of the pith respectively and the absence or the presence there of large intercellular spaces. Other things being equal, the younger the plant the more active in extension will the pith be.

Though the movement of tissues toward the centre in the cases mentioned generally indicates that the energy of the cortex is greater than that of the pith, it does not indicate that the cells of the young bundles are less energetical than those of the cortex. The bundles and the meristem between them also bear a part in closing up the intercellular spaces, but the amount of tissue there and the extension of cells are, compared with the cortex, so small that the effect is much less apparent.

A very striking effect of enclosing an internode in gypsum before primary growth has ended is the smaller definitive size attained by the parenchyma cells of the fundamental tissue. Such cells can from the nature of the case increase in length by gliding growth only, and this is shown by observation to be small. They can widen only to the extent of filling the intercellular spaces; and the room thus afforded varies with different species and with different ages in the same species. An internode for instance that has nearly completed its primary growth will have larger cells and larger intercellular spaces than one in which primary extension is farther from its termination.

It might be expected that the plant when it cannot produce so great an extent of cell-membrane as normal would make thicker membranes, and Wortmann¹ describes cases in which he has obtained this result. This Author wound the young stems of several plants with twine, thus stopping, he says, longitudinal extension, and radial also except for the growth made between the coils of the imperfectly fitting cord. An examination of structure after a few days' growth showed in the cortex of the constricted part smaller cells with thicker membranes than normal.

Pfeffer however, in the manuscript referred to, states that in the root-tips that had been encased in gypsum the cell-walls became no thicker. The 15 species of plants including scores of individuals

¹ Wortmann, Beiträge zur Physiologie des Wachsthum. Bot. Zeit. 1889, p. 286.

which have formed the material for the present work have in no case shown thicker walls within the casts, but on the contrary have shown in general thinner walls in pith and cortex. An individual of *Ricinus communis* an internode of whose stem was wound with twine as Wortmann wound his plants showed however after growing for some weeks in this condition a band of collenchyma in the cortex with walls vastly thicker than the same stem showed in normal positions. In this case the peripheral cells of the cortex had as in Wortmann's plants grown in among the coils of the twine.

In the plants that had made good growth after the gypsum casts were applied there was always in the vicinity of the upper limit of the cast thicker cell-walls than normal. This abnormal thickening could always be detected in pith and cortex cells, being strongest just above the limit of the cast and decreasing upward and downward from that position, reaching within a centimeter above the limit of the cast the normal condition, and a centimeter down into the cast the normal or a condition in which the cells had thinner walls than normal.

It is thus seen that in these plants the thickening of cell-membranes occurs within the cast only near the upper limit. Longitudinal sections show that the cells in the upper centimeter of the cast have undergone extraordinary gliding growth, while farther down in the cast such has not been the case. Just below the upper limit of the casts the walls of the cortical parenchyma that normally are nearly horizontal are inclined from the periphery toward the centre upward at an angle of 45° or more. Passing downward in the stem the inclination gradually becomes less till at the depth of about a centimeter the horizontal position is found. The outer cells of the stem have been held by the gypsum while the more internal by their efforts to elongate have undergone displacement upward. Thus it seems probable that the tension of tissues has been the stimulus for producing thicker walls. In the case of the *Ricinus* plant cited above and in those plants described by Wortmann it is not improbable that more than the normal tension arose in the cortex either by longitudinal growth of the part held by the band or by the uneven growth of the peripheral cells into the band, and thus stimulated the thickening of cell-membranes. That tension may stimulate such a formation is well known, especially from Pfeffer's¹ review of an unpublished research by Hegler.

¹ Pfeffer, R. Hegler's Untersuchungen über Einfluss von Zugkraft. Berichte d. k. sächs. Gesellsch. d. Wissensch. December 1891.

Within the casts the parenchyma cells of the fundamental tissue not only remain smaller and thinner-walled but their growing period and life-period are longer than normal. *Zea mais* and *Cucurbita pepo* which develop thick-walled sclerenchyma cells from their fundamental tissue show within the casts such cells slowly increasing the thickness of their membranes after corresponding normal cells in neighboring parts of the stems have ceased to grow. All plants in which the outer part of the pith parenchyma normally becomes thick-walled have shown, within the casts, this tissue always in a much thinner-walled state, but the membranes slowly increasing in thickness after the permanent condition in corresponding cells above and below the casts had been reached. Such plants are *Vicia faba*, *Cucurbita pepo*, *Melanthus major* and *Dahlia variabilis*.

All the plants that have been under experiment, except the one species *Ricinus*, have given positive evidence that the application of the cast before primary extension has ended prolongs the life-period of cells. *Ricinus* has shown within the cast dead crushed cells in the cortex as early as dead cortical cells were found outside the cast. But the normal cortex was then very spongy, the cells hanging together with but slight connections and certainly incapable of resisting such a pressure as was being exerted on the cortex within the gypsum. It is fair then to assume the possibility of the pressure from the region of the cambium crushing these cortical cells earlier than they would have died without such a crushing force. Thus *Ricinus* does not necessarily form an exception to the behaviour of the other plants examined in that they preserve longer the life of those cells which are not allowed to take their full size.

Except in the cortex of *Ricinus communis* all organs which normally become hollow or in which there is destruction of tissue have by the application of casts had such tissue longer preserved than in normal parts of the organ above and below the cast. This has proved true for the middle parenchyma of leaves of *Allium cepa*; for the central tissue of the petiole *Ricinus communis*; for the cells whose collapse forms the intercarinal canals of *Equisetum limosum*; for the cells which break down to form the air-canal in *Zea mais*; for the "pith" of the rhizome of *Triticum repens*; for the cortex of *Vicia faba*, *Laminum gargaricum*, *Urtica dioica*, and *Melanthus major*; for the pith of *Caltha palustris*, *Althaea tauriensis*, *Cucurbita pepo*, *Laminum gargaricum*, *Urtica dioica*, *Vicia faba*, *Archangelica sativa*, *Myrrhis odorata*, *Dahlia variabilis*, *Ricinus communis* and *Melanthus major*.

The effect of applying casts in prolonging the life of the pith

is deserving of especial attention. But before this is considered it will be well to discuss the process of normal cavity-formation together with its relation to primary and secondary growth.

3. The formation of cavity by the destruction of cells and its prevention by the use of gypsum casts.

De Bary¹ in his *Vergleichende Anatomie* has pointed out the intimate relation between schizogenous and lysigenous modes of origin of the cavity in the pith and mentioned the fact that one mode may replace the other in closely allied plants. De Bary's statement may be carried farther: One mode may replace the other in different individuals of the same species and in different internodes of the same plant. The fact is that there is no boundary between *schizogenous* and *lysigenous*. If for instance in *Vicia faba* one studies the cavity-formation in the stem at the distance of several internodes from the earth he finds the pith-cells forming a loose network which as primary extension progresses suffers many breaks, the cells becoming widely separated before any die. This beginning of cavity-formation is schizogenous. If now one traces cavity-formation in the epicotyl where the pith remains solid till primary extension has ended, he will find the cells dying before separating from one another, the mode being lysigenous. If the study is carried into the next internode above, where the cavity begins about the time that secondary growth begins, one finds the pith-cells shortly before cavity-formation with intercellular spaces larger than those appearing in the internode below, smaller than those in the internode above. The cells may die with or without any split appearing in the tissue, but if such split appears before any cells die its extent is very small. Naming the beginning of cavity-formation in such an internode either schizogenous or lysigenous is purely formal.

Urtica dioica, *Lamium garganicum*, and *Caltha palustris* become hollow as does *Vicia faba*, and the 2 former show in their lower internodes the same variation as pointed out for *Vicia*.

In *Archangelica*, *Myrrhis*, *Dahlia*, *Melianthus* and *Ricinus*, the early stages of cavity-formation are almost alike in the higher internodes where the stem becomes hollow before elongation has ended and in the lower internodes where the pith is solid for some time after secondary growth has begun. In the former case the inter-

¹ p. 223 et sq.

cellular spaces usually enlarge so as to form small clefts before any cells die: but these clefts lengthen to not more than the diameter of one to three cells before the bounding elements collapse. In the lower internodes where the pith cells all live for a time after secondary growth has begun, the cells usually collapse without more separation than that given by intercellular spaces at the angles of the cells. But examples are not wanting in which while the cells remain living the intercellular spaces in these internodes grow into clefts after secondary growth has been going on for some time. This extension of intercellular spaces can probably take place only by the contraction of cell-walls in the neighborhood. Cavity-enlargement can in the internodes where primary growth has ended proceed only by the shrinking and collapse of cells, while in other internodes the movement of all peripheral tissues from the centre carrying with them the enlarging outer pith-cells is an important factor. The difficulty in drawing a boundary between schizogenous and lysigenous origin of cavity that was pointed out in *Vicia* where the extremes are far apart is much greater in the last-named plants where the extremes are so little removed and where the passage of one form into the other is insensible.

Urtica dioica and *Dahlia variabilis* are good examples of the varying relation of cavity-formation to the completion of primary growth. In both of these species the stronger plants will show a cavity much earlier than the weaker ones. The average plant in the former when 6 or 7 internodes above the earth will show cavity in one of the middle internodes, but weak individuals have been found 12 internodes in length with 8 or 9 fully elongated and with solid pith everywhere. A strong *Dahlia* plant will have its middle internode hollow when 3 internodes are exposed above the soil, but weak plants may reach a height of 7 internodes with the lower 3 or 4 fully elongated and all without cavity. Thus in these and doubtless in many other plants, corresponding internodes in different individuals of the same species become hollow sometimes during primary growth, and sometimes not till weeks after secondary growth has begun. The early death of the cells at the centre of the stem would thus seem to be favored by the greater intensity and amount of primary extension in the more peripheral tissue; or in other words, before secondary growth begins, the less the extension of the peripheral relative to that of the central tissue, the longer will the latter live.

The experiments in encasing young stems in gypsum have led to the same conclusion. Experience has shown that if the pith is to be preserved much longer than it would live normally, the casts

must be applied before the cells have reached their full size. Thus, though in *Cucurbita pepo* several hypocotyls were put into casts, the middle of the pith was in no case kept alive to the time of the first examination, evidently because the cells had nearly reached their full development when the experiments were begun. In *Caltha palustris* the same condition was found; and one stem on being taken from the cast showed much of the pith dead but uncollapsed, demonstrating that the pith had here died without suffering the usual amount of tension from the cortex; for normally the pith is fully collapsed by the tearing which it suffers. In *Melianthus major* where it is impossible to apply a cast long before the middle pith-cells die, such cells have not been kept alive up to the time of the first examination from the cast though the growth of the shoot meanwhile was only 2^{cm} in length. In *Myrrhis odorata* precisely the same relations were found as mentioned for *Caltha*. In *Archangelica sativa*, *Lamium garganicum*, *Urtica dioica*, *Ricinus communis* and *Vicia faba* the entire pith has been kept alive for periods varying from 2 to 8 weeks after it would normally have died. The development in these periods can be found by referring to the record of the experiments. Suffice it to say *Dahlia* and *Vicia* have been longest in cast, the former for 4 months, the latter for 59 days without any dead pith appearing within the gypsum in any individual. *Dahlia* however had not grown very well in this time, but *Vicia* had reached the height of a meter and bore blossoms. In both species the pith had died above and below the casts, and it is certain that in the case of *Vicia* the pith had lived within the cast 8 weeks longer than it would normally have done. How much longer such a pith would live we have no means of knowing, but it does not seem impossible to think of it as continuing till the death of the plant in autumn, — not merely because it has not been injured by the growth of surrounding tissue, but it is possible that the plant as a regulatory means for meeting the exigency induced by the cast may put the pith to a new use, such as aiding in transport through the very narrow isthmus connecting the normal parts of the stem. Such a regulation would be similar to that discovered by de Vries¹ in a flower-stalk of *Pelargonium zonale* which bore a vegetative bud among the blossoms. The flower-stalk instead of dying in the usual way lived to function as a vegetative stem.

Many of these preparations that showed a living pith in the upper part of the casts presented more or less dead pith in the lower

¹ De Vries, Ueber abnormale Entstehung secundärer Gewebe. Jahrb. f. wiss. Bot. Bd. 22. (1891.)

part where it is certain no dead cells existed when the gypsum was laid around the stem. But in such cases as these the peripheral zone of living pith was greater within than outside the cast, and in those species where an entirely living pith was not preserved within the casts there was more living pith than in normal parts of the stems. Thus is it shown that the pith will live a short or long period beyond the usual time according as the casts are applied shortly or long before the cells would normally die.

It cannot be said that the pith in any of the plants under experiment would normally live much longer if not torn by the cortex. In all experiments where casts have been applied shortly before the time for tearing, the life of the pith has been prolonged but a few days.

4. Effect of gypsum casts on the growth of supporting and conducting tissue.

The most striking result obtained in these experiments is the lack of formation of mechanical tissue within the casts. It is self-evident that a stem whose growth is so confined that multiplication of cells cannot take place will not be able to form so much mechanical tissue as a normally growing stem. But a plant can be stimulated to increase more than normally the thickness of its cell-walls as has been demonstrated by the researches of Elfving¹ and Hegler². The former found that plant stems when forcibly bent showed the collenchyme on the convex side thicker-walled and on the concave side thinner-walled than normal. The latter by growing plants under a stretching force was able to call forth an increase of the various kinds of mechanical tissue accompanied by a greater thickness of cell-wall. It might be supposed that when within the plaster casts new cells could not be formed, the preexisting immature cells would become through regulation thicker-walled than usual. But the reverse is true. Cells formed before the casts are applied which normally become thick-walled remain thin-walled within the gypsum.

In none of the stems surrounded by gypsum has growth either by division or extension of cells been immediately brought to a standstill. As mentioned earlier, the room for this growth is first furnished by the intercellular spaces. After these are filled there

¹ Elfving, Zur Kenntniss der Krümmungserscheinungen der Pflanzen. Särtryck ur Öfersigt af Finska. Vet. Soc. Förhandlingar. Helsingfors 1888.

² Hegler [in the review by Pfeffer previously mentioned].

has been in most cases a crushing of primary vessels. In the space thus made the meristem during the first few weeks slowly produces new cells and the more vigorous of the immature cells gradually expand. Usually with this slight growth a few cells begin to show thickening walls. If the pith should not be resistant, as it is not in *Cucurbita*, or if the cortex should be crushed, as in *Ricinus*, farther growth will take place and there will be more thickening of cell-walls. But even under such conditions if the pressure is great the amount of mechanical tissue will be far below normal.

Several plants that have reached a large growth after the cast was applied and thus have made a great transpiring surface above the position of the cast have shown a very slow but gradual thickening of xylem cells without showing an increase in size of such cells. Such plants are *Vicia* and *Ricinus*. It is possible in these cases that transpiration has been the stimulus for such growth. If as Kohl's¹ experiments seem to show the amount of thick-walled tissue is increased or decreased with the increase or decrease of transpiration, we should expect to find a corresponding regulation in such a narrow neck of stem as that within the cast where the cross-section is often no more than one-fourth that of the same stem in normal positions. Yet such an effect by transpiration is not great enough in these experiments to allow at present a positive statement to be made regarding it.

It must not be supposed that the enforced small size of cells is the one sufficient condition for the thinness of wall. As mentioned before, the behaviour of tissues within the casts, but within a centimeter of the limits, is quite different from what has just been described for the middle of the cast. In those stems where the casts have been applied before primary growth has ended and where such stems have made a fair subsequent growth, the mechanical tissue in the neighborhood of the limits of the casts has always been abnormally great. Just outside the limits of the cast where the stem has nearly reached its normal thickness the amount of such thick-walled tissue is greater than anywhere else in the stem. Just within the limits of the cast the amount of such tissue is greater than elsewhere in the cast and greater than occurs in tissues of equal development in normal parts of the stem. As an illustration to make the meaning of the last more clear, it may be said that in the middle of the part of the stem within the cast the xylem cells among the vessels and the pith-cells bounding the bundles will be

¹ Kohl, Die Transpiration der Pflanzen. Braunschweig 1886.

found thinner-walled than in normal parts of the stem, while within the cast but near its limit the corresponding cells though no larger than farther within the cast will be thicker-walled than such cells in normal parts of the stem. It should be added also that the abnormal increase of mechanical tissue is greater at the upper limit of the cast than at the lower. The difference is probably to be accounted for from the fact that primary growth when the cast was applied was more nearly ended at the lower limit than at the upper, and hence at the upper as indicated by the evidence of gliding growth there has been more tension of tissue. Tension as a stimulus to formation of mechanical tissue has been previously discussed in this paper.

All kinds of mechanical tissue found in the plants that have been under experiment have remained more or less undeveloped within the casts: the collenchyma of *Archangelica* and *Myrrhis*; sclerenchyma of *Triticum*, *Zea*, *Caltha* and *Cucurbita*; hard bast, xylem elements and pith of all plants studied. In all the plants with the possible exception of *Urtica*, the cells which normally become hard bast are the last to show any thickening of membrane within the cast.

As was found true for the parenchyma cells, so also the mechanical cells retain longer within than outside the cast the power of growth. Yet eventually the cells that normally become mechanical must within the cast pass into their mature stage without reaching their normal development. When a segment of a stem during primary growth is confined in gypsum for several weeks, the remainder of the stem growing meanwhile, the confined segment will not elongate on being released from the cast, as has been proved experimentally in several cases. The cells have matured without reaching their normal length.

With the possible exception of *Triticum*, *Zea* and *Melanthus* all plants that produced enough mechanical tissue within the cast to allow a comparison to be made showed in cross-section the elements of such tissue smaller than normal elements. Thus in horizontal diameter also, the mature condition may be reached with an extension less than normal. This conclusion was reached by de Vries and Krabbe for tracheids and vessels of woody plants; it would seem to be general for all cells.

It is not improbable that cells normally mechanical will mature at a thinner-walled condition than usual, though the study in this direction has been conclusive in but 2 cases. It is difficult to determine from observation of sections when cells within the con-

stricted parts of the stems have lost their ability to grow. In *Melianthus* however which forms periderm within the cast as outside of it, except that its formation is a little delayed within the cast, the xylem parenchyma that is formed before the death of that part of the cortex outside the periderm remains thinner-walled than the cells formed subsequently. The death of the cortical cells releases the whole pressure of the cast. In *Vicia* the same result has been brought about by removing several casts after an application of several weeks. In these cases also the elements of the inner part of the xylem zone have remained thinner-walled than those formed subsequently to the removal of the cast.

De Vries found that under pressure the number of vessels proportionate to the other xylem elements formed was reduced. In the present researches the like can be proved true for *Melianthus* only. But the use of casts is not favorable to the study of the question, since there is generally too little growth within the confined part to allow one to judge whether the proportion between number of vessels and of other elements has changed or not.

The proportion of phloem to xylem cannot be seen to change in the part within the cast. The result is therefore that there is but little phloem produced in that position.

5. Changes outside the casts.

The quite general effect in inducing a resting period in young plants by the application of casts has been given on page 36. Other effects not so general are indicated at the beginning of the description of experiments for each plant species.

One other feature has presented itself in the plants *Cucurbita*, *Althaea*, *Vicia*, *Dahlia* and *Ricinus*. Always in all that part of the stem below the cast the development is weaker than that above. The thickness of stem is less because the activity of the cambium is less. In the 5 plants mentioned 4 are those that have grown well and for a long time within the casts. *Melianthus* is the only other plant that has thrived and grown for a long period within the cast, and in this the early death of the cortex soon made the cast ineffective. Thus it is probable that the diminished growth below the cast would be general.

Neither the presence nor the absence of a weight can be ascribed as the cause of this phenomenon since it appears in cases where the stem below the cast bears the whole weight of gypsum and stem above, and also in cases where the weight of both these parts is

removed by external support. It is possibly caused by poor nutrition, the transport from above downward being intercepted by the small amount of conducting tissue within the cast.

IV. RESULTS.

From the foregoing experiments, the following results have been obtained:

1. When all the usual conditions of growth are present, meristematic and young tissue, by a mechanical force resisting extension, may be held for an undetermined period in a condition amounting to rest without losing their functional capability.

2. The life-period of all cells may be prolonged by preventing their full extension through mechanical resistance.

3. Organs in which the death of cells accompanies the cavity-formation during primary growth will remain longer without dead cells if the extension of the stem is checked; and the earlier the extension is checked the longer will be the life-period of the cells.

4. Under pressure all elements will finally mature at a smaller size than normal.

5. A mechanical resistance so applied as to prevent enlargement and reduce tension will lessen the formation of mechanical tissue.

6. A mechanical resistance so applied as to prevent enlargement but to increase tension will increase the amount of mechanical tissue.

7. Of mechanical tissues growing under pressure the hard bast will remain longest thin-walled.

8. When the resistance from the centre is not great and the plant cannot extend radially, the tissues will grow toward the centre.

9. When a segment of a stem through mechanical resistance is kept from enlarging by secondary growth, the part of the stem below the constriction will in many cases grow less rapidly than that above.

VITA.

Born in Flint, Michigan, U. S. A., May 11, 1858, of protestant parents, the father being a farmer.

Was graduated from the Flint High School at the age of 21 in the year 1879, having pursued there the *classical* course till the last semester, when instead of continuing in Latin and Greek the natural history studies were taken up.

Subsequently for one year was teacher in the common schools, then for 7 years teacher in the Michigan School for the Deaf, the last 2 years head teacher.

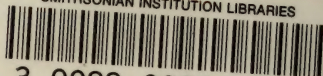
Was married in 1884 to Susan Eastman, also of protestant parents.

In 1887 entered the Michigan University, receiving on examination advanced standing so that the degree B. S. was conferred 3 years afterward.

In 1890, was appointed *Instructor in Botany* in the same institution and continued in that position for 2 years, — till the summer of 1892.

In the year 1888, published in the *Botanical Gazette* a research on the *Spore-dissemination of Equisetum*; in 1891 in the *Journal of Mycology* a short article on the *Discovery of a Perennial Mycelium in Caeoma nitens*. During 1891 and 1892 was engaged in a work, later to be published by the U. S. Government, on Economic Fungi, and in this period completed the study and drawings for the morphology of 12 species of such Fungi.

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